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(54) Title: MONOCLONAL ANTIBODY TO CELL SURFACE PROTEIN OF THE BACTERIUM NEISSERIA MENINGITIDIS

(57) Abstract

This invention relates to a monoclonal antibody (Mab) directed against a cell surface protein of *Neisseria meningitidis*, a hybridoma cell line producing said antibody, and the use of such an antibody to detect the bacterium *Neisseria meningitidis* or to detect antigens of *Neisseria meningitidis*.

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MONOCLONAL ANTIBODY TO CELL SURFACE PROTEIN OF THE BACTERIUM NEISSERIA MENINGITIDIS

BACKGROUND OF THE INVENTION

The present invention involves a monoclonal antibody (Mab) with the specificity for a 20,000 dalton cell surface protein of Neisseria meningitidis, a cell line that produces said antibody, and the partially purified 20,000 dalton cell surface protein.

N. meningitidis is one of the leading causes of community-acquired bacterial meningitis, causing 19.6% of reported cases in the United States between 1978-1981. Meningococcal meningitis is most prevalent among infants between 6-12 months and adolescents. In addition to meningococemia, other less commonly associated diseases such as, conjunctivitis, sinusitis, endocartitis and primary pneumoniae can also occur.

Neisseria meningitidis bacteria are carried in the nasopharynx of 10-15% of healthy individuals. In spite of the high carriage rate, its presence does not necessarily imply infection. However, if N. meningitidis is isolated in cerebral spinal fluid or blood culture, its detection is significant.

Detection of this bacteria at an early stage is essential to facilitate treatment of the infection. Thus, it is important to possess the ability to identify whether N. meningitidis is present in a patient and to follow the effect

of antibiotic treatment on the bacteria. As available immunoassays for N. meningitidis antigen detection have shown lack of specificity and/or sensitivity, there remains the need for an improved method of such detection.

N. meningitidis is a gram negative bacteria. Proteins located on the cell surface of many gram negative bacteria have, in the past, been used in typing and immunoprotective studies. There are a large number of N. meningitidis strains, and there are many cell surface proteins associated with N. meningitidis. This has made identification of a common but exclusive cell surface antigen difficult. However, Mab technology has provided researchers with tools to accurately analyze the cell surface components of N. meningitidis. In addition, N. meningitidis proteins are of interest to the epodemiologists as they may provide for vaccines against the bacteria.

Meningococcal vaccines have been developed using capsular polysacharrides. One particular quadravalent vaccine incorporates polysacharride antigens of serogroups A,C,W and Y, meningococci that are responsible for less than 49% of meningococcal disease in the United States. The most prevalent N. meningitidis serogroup is serogroup B. No capsular polysacharride vaccine is available for serogroup B as it is poorly immunogenic. In general, polysacharride vaccines are poorly immunogenic in infants because they are T-lymphocyte independent antigens which are inefficient at

inducing an immunologic memory. Furthermore, no cross protection between serogroups occurs. Thus, there remains the need for an improved meningococcal vaccine.

It follows then, that there remains a need for at least two products relating to N. meningitidis. The first is a rapid, specific, and sensitive diagnostic test for all strains of N. meningitidis, that does not give false positive results. What is optimally desired is a Mab that will recognize a cell surface antigen that is universally present in most, if not all, strains of N. meningitidis and, at the same time does not recognize other organisms or material which may be found in conjunction with N. meningitidis. Secondly, it is desirous that the Mab and said 20,000 dalton protein be used in research towards development of an improved vaccine.

Summary of the Invention

The present invention involves a monoclonal antibody (Mab) that is reactive with an epitope (an antigenic determinant of known structure) of a proteinaceous surface component of the bacterium N. meningitidis with the said antibody being reactive with said antigen in at least 99% of strains of N. meningitidis.

It is preferred that such Mab is reactive with an epitope of a proteinaceous cell surface component of the

bacterium N. meningitidis, particularly a protein of approximately 20,000 daltons.

An additional aspect of this invention involves a cell line capable of producing a Mab that is reactive with an epitope of a proteinaceous cell surface component of the bacterium N. meningitidis with said epitope being present in at least 99% of strains of said bacterium.

It is preferred that said cell line be capable of generating a Mab that demonstrates specificity for an epitope of a proteinaceous cell surface component of bacterium N. meningitidis. It is preferred that said cell line is a hybridoma cell line specifically a hybrid of a mouse spleen cell and an immortal myeloma cell.

A further aspect of this invention provides a diagnostic method to identify, type, and/or detect the presence of the bacterium N. meningitidis or its antigens, with such methods (a) causing the test sample to come into contact with said Mab; and (b) observing whether cell-labelling or agglutination occurs, indicating the presence of N. meningitidis or an antigen of N. meningitidis.

It is preferred that such a method involves a Mab that is reactive with an epitope of a proteinaceous cell surface component that is present in a least 99% of the known strains of N. meningitidis. It is additionally preferred that

the said label is chosen from a radio-label, florescent label, colloidal gold label, biotin label or enzyme label. This method could also be employed to detect infection of N. meningitidis in patients.

An additional feature of this invention provides a significantly purified form of the said proteinaceous cell surface component of the bacterium N. meningitidis having an epitope present in at least 99% of the strains of the said bacterium. A preferred embodiment of this feature is a 20,000 dalton protein or fragment thereof containing such an epitope. It is to be preferred that an epitope of said component or part thereof is present in more than 99% of the strains of N. meningitidis, and is only present in said bacterium.

I have generated a Mab that specifically recognized an epitope of a proteinaceous cell surface component of the N. meningitidis common to 99% of all strains of said bacterium. The use of this Mab for immunodiagnosis and typing is disclosed.

Detailed Description of the Invention

The production of a monoclonal antibody directed against a common protein of Neisseria meningitidis.

The Strains of Bacteria and Culture Conditions

N. meningitidis strains were obtained from clinical isolates from the following: Children's Hospital of Eastern Ontario (CHEO), Ottawa; Laboratoire de la Santé Publique de Québec; Sainte-Anne de Bellevue; Trinidad; Provincial Laboratory Of Public Health of Nova Scotia, Halifax; Provincial Laboratory of Public Health Of Saskatchewan, Regina; Montréal Children's Hospital (MCH), Montréal; Laboratory Centre for Disease Control (LCDC), Ottawa. N. meningitidis was grown on chocolate agar plates supplemented with 1% ISOVITALEX® (BBL, Cockeysville, Md) overnight at 37°C, in atmosphere containing 5% CO_2. The resulting cultures were stored in brain heart infusion broth containing 20% glycerol at -70°C.

Outer Membrane Preparation

The extract of the outer membrane proteins from the bacteria was performed using the method previously described by Johnston et al., J. Exp. Med. vol. 143, 741-758 (1976). Whole cells were suspended in lithium chloride buffer (200 mM lithium chloride, 100 mM lithium acetate, 10 mM EDTA, pH 6.0), transferred to a 250ml Erlenmeyer flask containing 3-5 mm glass beads and shaken 300 rpm in G24 Environmental incubator shaker for 2 hours at 45°C. The suspension was centrifuged at 8000 rpm for 20 minutes using Sorvall SS-34® fixed angle rotor with R_{max} = 10.70 cm. Collected supernatant was transferred to

a rigid wall polycarbonate tube and ultracentrifuged at 35.0k (35,000 rpm) for 2 hours at 10°C using a 50.2 Ti rotor (Beckman®). Supernatant was discarded and the pellet resuspended in 1 ml of phosphate buffer saline (PBS). Protein content was determined by method described by Lowry et al., J. Biol. Chem., vol. 193, 265-278 (1951).

Using protein preparation and a standard of 1 mg/ml BSA, prepare 6 volumes of each ranging from 0 to 100 μl were prepared. To each tube, 2 ml of 2% Na_2C0_3 in NaOH (1N) was added vortexed and incubated at 56°C for 2 hours. Equal volumes of $CuS0_4 - 5H_20$ (1%) and K-Na tartrate (1%) (i.e. 40 μl of each, was added and incubated for 20 minutes at room temperature). To the solution 200 μl Folins reagent (1N) was added and mixed. After 30 minutes incubation, the OD_{750nm} was read.

Immunization of Mice

A Balb/c mouse was inoculated interperitoneally with 10 μg of N. meningitidis strain 604A outer membrane proteins from lithium chloride extraction, combined with complete Freund's adjuvant. Two weeks later, the mouse was reinjected intraperitoneally with 10 μg proteins in incomplete Freund's adjuvant. Four days prior to hybridoma production, a third injection of 10 μg N. meningitidis strain 2441C proteins from the lithium chloride extraction was given intraperitoneally

without adjuvant. Serum was obtained from the immunized mouse by cardiac punctures before spleen removal.

Fusion Procedure

Hybridomas were produced according to a modification of the methods described by Fazekas De St. Groth and Scheidegger, J. Immunol Methods, vol. 35, 1-21 (1986). Spleen cells from immunized mouse and nonsecreting, HGPRT deficient, mouse myeloma cells SP2/0 were fused in a ratio 10:1 in Dulbecco modified Eagle's medium (DMEM, Flow Laboratories, Mississauga, Ontario, Canada) containing 50% (w/v) polyethylene glycol 1540 (Kodak, Toronto, Ontario). The fused cells (0.1 ml, 1.5 X 10^5 cells/ml) were portioned into 96-well tissue culture plates (GIBCO BRL, Burlington, Ontario) which contained a feeder layer of 4 X 10^3 murine peritoneal exudate cells (marophages). The suspensions of cells were grown in DMEM that were supplemented with 20% fetal calf serum (Gibco), 2mM L-glutamine (Sigma Chemical Co., St. Louis, Mo.), and 50 μg/ml gentamicin (Sigma) in the presence of hypoxanthine, aminopterin and thymidine (HAT) selection medium. All cultures were checked on day three for the presence of clones and the medium was changed on day eleven. Supernatants of wells containing growing cells that were tested on day twelve by the ELISA for Mab directed against N. meningitidis antigens. The cells that were producing antibody were subcloned through limiting dilution. Subclones that were selected were grown whether as ascities according to the

method of Brodeur et al. J. Immunol Methods, vol. 71, 265-272 (1984) or in vitro for freezing in liquid nitrogen.

Immunoglobulin Class Determination

The supernatant from the cells producing antibodies were tested against affinity purified anti-mouse immunoglobulin (Southern Biotech) using the ELISA method.

Enzyme-Linked Immunosorbent Assay (ELISA) Procedure

Screening of the resulting supernatants for the Mabs directed against N. meningitidis was performed as described by Brodeur et al., J. Med. Microbiol, vol. 15, 1-9 (1982). The antigen (0.1 ml) containing 0.75 μg protein in 0.05M carbonate buffer at pH 9.6 was portioned into each well of a high-binding microtiter plate (Flow). The plate was incubated overnight at room temperature to permit the adsorption of the antigen. The plate was then washed with PBS containing 0.02% Tween-20 (Sigma) and 150 μl of 0.5% bovine serum albumin (BSA, Sigma) in PBS was added to each well. The plate was incubated at 37°C for 30 minutes. The BSA was discarded and the plate was washed and the test supernatants were added. The positive control was a standard serum. After a one hour incubation at 37°C, the plate was washed three times. This was followed with the addition of 0.1 ml alkaline phosphotase-conjugated goat anti-mouse immunoglobulins (BRL) diluted 1:3000 in PBS containing 3% BSA. The plate was incubated at 37°C for an

additional 1 hour. The plate was then washed and 0.1 ml of a 10% diethanolamine solution (pH 9.8), containing 1 mg/ml p-nitrophenylphosphate (Sigma) was added. The plate was allowed to stand for 1 hour. The absorbance was then determined spectrophotometrically using a DYNATECH® microplate reader MR 600 at 410 nm. Readings greater than 0.1 were scored as positive, indicating the presence of antibodies directed against N. meningitidis.

SDS-Polyacrylamide Gel Electrophoresis (PAGE)

Resolution of the proteins were achieved through electrophoresis on sodium dodecyl sulfate (SDS) 0.75 mm thick slab mini gels according to the method described by Laemmli, Nature, vol. 227, 680-685 (1970). A 12% acrylamide (Bio-Rad, Laboratories, Mississauga, Ontario, Canada).) resolving gel and a 4.0% stacking gel were utilized. Cell lysates used on the gels were prepared by lithium chloride extraction. Lysates were mixed with sample buffer (62.5 mM Tris-HCl) pH 6.8, 1% (v/v) glycerol, 2% (w/v) SDS, 0.5% (v/v) 2-mercaptoethanol and 0.5% (w/v) bromophenol blue and heated for 4 minutes at 100°C. Aliqouts of 15 μl containing 5 μg of protein were applied to each gel lane. Electrophoresis was carried out at 100 V constant voltage until the bromophenol blue tracking dye entered the separating gel. At this time, the voltage was then increased to 200 V. The gels were strained with Coomassie blue dye and then destained following the method of Weber and Osborn, J. Biol. Chem., vol. 244,

4406-4412 (1969). The protein standards (with respective MW) used were: Bovine serum albumin (66,200), ovalbumin (45,000), carbonic anhydrase (28,000), Soybean Trypsine Inhibitor (20,100), and alpha-lactalbumin (14,200) (Bio-Rad Laboratories, Mississauga, Ontario, Canada).

Immunoblotting Procedure

The proteins were transferred electrophoretically from the SDS-PAGE gel to nitrocellulose paper (Bio-Rad) by the method described by Towbin et al., Proc. Nat. Acad. Sci., vol. 76, 4350-4354 (1979). A constant current of 35 mA was applied to the gel-nitrocellulose paper sandwich for 1 hour. This was done in an electroblot buffer of 25mM Tris-HCl, 192 mM glycine and 20% (v/v) methanol at pH 8.3. The proteins transferred onto the blot were either stained with amido black or detected by an enzyme immunoassay. The detection of bacterial antigens was performed by soaking the paper in PBS solution containing 1% milk for 30 minutes in order to block non-specific protein binding sites. The paper was then incubated with mouse hyper-immune sera at 37°C for 1 hour. The sheet was washed three times with PBS followed by a 1 hour incubation at 37°C with peroxidase-conjugated goat anti-mouse immunoglobulins (Cappel, Cochranville, Pa.) diluted 1:1000 in PBS containing 3% BSA. The sheet was once again washed three times and the blots were soaked in a solution of o-dianisidine prepared as described by Towbin et al., supra.

Surface Accessibility Assay

A radioimmunoassay was used to determine whether Mabs were directed against cell surface exposed epitopes of various strains of N. meningitidis. Strains were grown on Columbia blood agar plates overnight at 37°C in a 5% CO_2 humidified atmosphere. The bacteria were suspended in PBS, equal volumes dispensed into 2 ml tubes, centrifuged to pellet the cells and the supernatants was discarded. Culture supernatants containing Mabs were incubated with resuspended live bacterial cells for 2-3 hours at 4°C. The bacteria were then washed twice with PBS, incubated with ^{125}I-labelled goat anti-mouse IgG (DuPont) for one hour, washed and pelleted.

The bacterial cell-bound ^{125}I was counted using a 1282 Compugamma (LKB Instruments Inc.). The means of triplicate determinations were calibrated and background reaction using negative controls were subtracted.

Dot-Enzyme Assay

A dot-enzyme assay was used for a quick method of screening several Mabs against a large number of N. meningitidis strains. The strains were grown on chocolate agar plates overnight. A small amount the suspension, approximately 50 μl, was applied to a nitrocellulose paper using a DOT-BLOT apparatus (Bio-Rad Laboratories, Massasauga, Ontario, Canada). The dot nitrocellulose paper was then

processed following the procedure described in the immunoblotting procedure.

Properties of Monoclonal Antibodies

More than 800 hybrid clones were obtained by fusing sensitized mouse spleen cells with SP2/0 cells. The screening for the Mabs in the hybridoma culture supernatants was performed by ELISA, utilizing the homologous immunizing N. meningitidis strain 604A and heterologous strain 608B lithium chloride extract as the coating antigens. Every positive hybrid clone supernatant was further tested against several other strains of N. meningitidis. Eleven hybridoma cell lines that demonstrated different patterns of reactivity in ELISA were obtained (see Table I).

TABLE 1 Characterization of Monoclonal Antibodies Directed Against N. meningitidis Antigens.

	Clone	Immunoglobulin Class/subclass	O.D.at 410 mm	Molecular weight of Antigen recognized in kD	Surface Access.	Specificity to N. meningitidis
1)	1A-3	IgM	0.547	90		yes
2)	2D-6		0.696	70		non-specific
3)	3D10	IgG	0.041	30		specific to sero-group B,C + W
4)	3F11	IgG1	0.673	70	no	non-specific
5)	4G75	IgG1	0.215	14		non-specific
6)	6G7	IgG2A	0.244	20	yes	yes
7)	11A7		0.002			specific sero group E only
8)	11G11		0.941			non-specific
9)	15F9	IgG2A	0.111	20	yes	yes
10)	16B9	1gG	0.819	70	no	non-specific
11)	16F7		0.892	70		non-specific

The monoclonal antibody from the clone 15F9 was very specific to all the strains of N. meningitidis. 15F9 was subcloned twice by limited dilution and the class and subclass of the Mab were determined using affinity purified anti-mouse immunoglobulin in an ELISA. This clone was then identified as 15F9/D7/H2 and the Mab was given the official designation of Nm-2.

Identification of Antibody-Specific Epitopes on the Antigen

The Western immunoblotting technique was used to ascertain the specific antigen to which each Mab binds. The mouse hyperimmune serum that was used as positive control, detected all the major proteins present in strains of N. meningitidis.

Nine of the eleven Mabs reacted with antigens transferred from the SDS-PAGE to nitrocellulose paper. The remaining 2 Mabs were not tested. Five different antigens were recognized by the Mabs with apparent molecular weights of 90,000, 70,000, 30,000, 20,000 and 14,000 daltons.

Binding Properties of Monoclonal Antibody Nm-2

To determine whether clone 15F9 was directed against the cell surface exposed epitope of the 20,000 dalton protein, or part thereof, hybridoma culture supernatants containing the Mabs were screened by radioimmunoassay.

Fewer than 3,000 cpm were obtained using culture media as a negative control. Supernatant containing the Mab Nm-2 showed counts much greater than negative control containing an unrelated Mab (Table II), indicating that the component is surface accessible.

Table II: Binding Properties of Monoclonal Antibody Nm-2

Bacterial Strain	CPM of bacterial cell-bound ^{125}I		
	N. meningitidis clones[1]		Negative Control[2]
	15P9	3F11	
N. meningitidis 604A	29003	1169	944
N. meningitidis 608B	23093	264	0
N. meningitidis 2241C	21273	0	133
N. meningitidis 2S E	12355	769	0
N. meningitidis W135	22063	1258	181
N. meningitidis 247X	24289	584	0
N. meningitidis Stat Y	20125	98	713
N. meningitidis Stat Z	22699	0	62
N. cinerea	35	472	0

Note: Data represents means of triplicate determinations

1 Background CPM due to culture media was subtracted

2 Negative control anti-*streptococcus pneumoniae* Mabs.

Specificity of Monoclonal Antibody Nm-2

The initial ELISA characterization showed Nm-2 reacted only with *N. meningitidis* strains. A dot-enzyme immunoassay was used for a rapid method of screening this Mab against numerous bacterial strains. The Mab Nm-2 reacted specifically with 233 *N. meningitidis* strains and only cross reacted with one strain of *Straphylococcus aureus* and one strain of *N. lactamiea* (Table III).

Table III: Specificity of Monoclonal Antibody Nm-2

Bacterial Strains	Reactivity by DOT-blot[1]
N. meningitidis	233/236
N. gonorrhoeae	0/49
non-pathogenic Neisseria	1/22[2]
Streptococci sp.	0/30
E. coli	0/2
H. influenzae	0/1
B. catarrhalis	0/4
Branhamella sp.	0/1
Bacillus sp.	0/1
Bronchi	0/1
B. pertussis	0/1
K. pneumoniae	0/1
P. aeriginosa	0/1
S. aureus	1/1[3]
S. epidermidis	0/2

1 Number of positive/Number of strains

2 Positive is N. lactamica 81-193 from LCDC, Ottawa, Ontario

3 Positive strain is S. aureus C723/90 from CHEO, Ottawa, Ontario

Note: The three N. meningitidis that are not recognized by the DOT-assay are:

i) N. meningitidis serogroup B, C31/87, from CHEO, Ottawa, Ontario

ii) N. meningitidis serogroup B, C1568/84, from CHEO, Ottawa, Ontario

iii) N. meningitidis serogroup A, 30490, L-hip aspirate, from MCH, Montréal, Québec

I claim:

1. The Nm-2 monoclonal antibody which specifically binds to a cell surface-accessible protein antigen of the bacterium Neisseria meningitidis.

2. The 15F9 cell line that produces a monoclonal antibody that specifically binds to a cell surface-accessible protein antigen of the bacterium Neisseria meningitidis with said antigens being present in at least 233 out of 236 of the strains of said bacterium.

3. The hybridoma cell line of claim 2, formed by fusing immunized mouse spleen cells and mouse myeloma SP2/0 cells, that produces the Nm-2 monoclonal antibody that specifically binds to a cell surface accessible protein antigen of Neisseria meningitidis.

AMENDED CLAIMS

[received by the International Bureau on 14 December 1993 (14.12.93); original claims 1-3 replaced by amended claims 1-26 (4 pages)]

1. An antibody or fragment thereof that specifically binds to a protein with a molecular weight of approximately 20,000 daltons present on greater than 50% of known strains of *Neisseria meningitidis*.

2. The antibody or fragment of claim 1 that specifically binds to about 99% of known strains of *N. meningitidis*.

3. The antibody or fragment of claim 1 which is a monoclonal antibody or fragment thereof.

4. The monoclonal antibody or fragment of claim 1 which is of murine origin.

5. The monoclonal antibody or fragment of claim 4 which is of an IgG isotype.

6. The monoclonal antibody or fragment of claim 5 which is Nm-2.

7. A hybridoma which produces a monoclonal antibody that binds to greater than 50% of known strains of *N. meningitidis*.

8. The hybridoma of claim 7 which is 15F9 (ATCC No. HB 11431).

9. An isolated antigen or fragment thereof which is immunologically accessible on greater than 50% of known strains of *N. meningitidis*.

10. The isolated antigen or fragment of claim 9 which is immunologically accessible on about 99% of known strains of *N. meningitidis*.

11. The isolated antigen or fragment of claim 9 in which immunological reactivity is determined using an agglutination assay, an ELISA, a RIA, an immunoblotting assay, a dot-enzyme assay, a surface accessibility assay, or a combination of these assays.

12. The isolated antigen or fragment of claim 9 which is a protein.

13. The protein of claim 12 which has a molecular weight of about 20,000 daltons.

14. A method for isolating the antigen of claim 9 comprising:
    a) isolating a culture of *N. meningitidis* bacteria,
    b) isolating an outer membrane portion from the culture of the bacteria; and
    c) isolating said antigen from the outer membrane portion.

15. A method for isolating the antibody of claim 1 comprising:
    a) introducing a preparation of *N. meningitidis* into a mammal, and
    b) isolating serum from the mammal containing said antibody.

16. A method for isolating the monoclonal antibody of claim 4 comprising:
    a) introducing a preparation of *N. meningitidis* to antibody producing cells of a mammal,
    b) fusing the antibody producing cells with myeloma cells to form hybridoma cells, and
    c) isolating said monoclonal antibody from the hybridoma cells.

17. The method of claim 16 wherein said antibody producing cells are murine spleen cells.

18. The method of claim 16 wherein said myeloma cells are murine SP2/O cells.

19. The method of claim 16 wherein the preparation of *N. meningitidis* is selected from the group consisting of a whole cell extract, a proteinaceous extract, and a membrane preparation.

20. A vaccine comprising isolated antigen or fragment of claim 9.

21. A method for preventing infection of a patient by *N. meningitidis* comprising the administration of a prophylactically effective amount of the vaccine of claim 20.

22. A vaccine comprising antibody or fragment of claim 1.

23. A method for treating a patient infected with or suspected of being infected with *N. meningitidis* comprising the administration of a therapeutically effective amount of the vaccine of claim 22.

24. A diagnostic aid for the detection of *Neisseria* antigen in a biological sample containing or suspected of containing *Neisseria* antigen comprising:

a) isolating the biological sample from the patient;

b) incubating the antibody or fragment of claim 1 with the biological sample to form a mixture; and

c) detecting specifically bound antibody or bound fragment in the mixture which indicates the presence of *Neisseria* antigen.

25. A diagnostic aid for the detection of antibody specific to *Neisseria* antigen in a biological sample containing or suspected of containing said antibody comprising:

a) isolating the biological sample from the patient;

b) incubating the antigen or fragment of claim 9 with the biological sample to form a mixture; and

c) detecting specifically bound antigen or bound fragment in the mixture which indicates the presence of antibody specific to *Neisseria* antigen.

26. A method for the detection of *N. meningitidis* in a patient comprising:

a) labeling the antibody or fragment of claim 1 with a detectable label;

b) administering the labeled antibody or labeled fragment to the patient; and

c) detecting specifically bound labeled antibody or labeled fragment in the patient which indicates the presence of *N. meningitidis*.

Applicant's or agent's file reference number 1907t J104	International application No. PCT/US 9[illegible]/08048

INDICATIONS RELATING TO A DEPOSITED MICROORGANISM

(PCT Rule 13*bis*)

A. The indications made below relate to the microorganism referred to in the description on page _____, line _____

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Date of deposit	Accession Number
04 August 1993	HB 11431

C. ADDITIONAL INDICATIONS *(leave blank if not applicable)* This information is continued on an additional sheet ☐

D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE *(if the indications are not for all designated States)*

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US93/08048

A. CLASSIFICATION OF SUBJECT MATTER

IPC(5) :C07K 15/28; C12N 5/12
US CL :530/388.4; 435/240.27

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 530/388.4; 435/240.27

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

DIALOG, APS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Infection and Immunity, volume 43, No. 3, issued March 1984, J.G. Cannon et al., "Monoclonal Antibody that Recognizes an Outer Membrane Antigen Common to the Pathogenic Neisseria Species but not to Most Nonpathogenic Neisseria Species", pages 994-999, see entire document.	1
Y	Infection and Immunity, Volume 50, No. 2, issued November 1985, B.R.Brodeur et al., "Protection against Infection with Neisseria meningitidis Group B Serotype 2b by Passive Immunization with Serotype-Specific Monoclonal Antibody", pages 510-516, see entire document.	2-3

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be part of particular relevance

"E" earlier document published on or after the international filing date

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Date of the actual completion of the international search
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